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INVESTIGATION OF THE INTRACELLULAR DISTRIBUTION OF ZINC AND ACID PHOSPHATASE ACTIVITY BY A COMBINATION OF HISTOCHEMICAL REACTIONS IN A GIVEN SECTION

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(Issledovaniye vnutrikletochnogo raspredeleniya tsinka i aktivnosti kisloy fosfatazy putem sochetaniya gistokhimicheskikh reaktsiy na odnom i tom zhe sreze)

I. V. Toroptsev and V. A. Yeshchenko

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INVESTIGATION OF THE INTRACELLULAR DISTRIBUTION OF ZINC AND ACID PHOSPHATASE ACTIVITY BY A COMBINATION OF HISTOCHEMICAL REACTIONS IN A GIVEN SECTION

I. V. Toroptsev and V. A. Yeshchenko*

ABSTRACT. Methods are presented for a successive detection of zinc and acid phosphatase activity in a single section, enabling comparison of the intercellular distribution of the two components. Frozen sections are used for this purpose, fixed in an 0.01% acetone solution of one substance of a series of 8-(arensulfonylamino)-quinolines or paraffin sections of tissues fixed according to Timm.

Zinc is required for the activity of a number of enzymes (Vallee, 1959; Voynar, 1960, Petkov and Galabova, 1968, Lazaris and Karlinskiy, 1970). Their topochemical interrelationships have not yet been studied, however. The latter requires methods allowing comparison of the intracellular distribution of zinc and enzyme in a single section. The purpose of the present investigation was to develop such methods.

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The dithizone (Okamoto, 1942, Maske, 1953; Yeshchenko and Sukhanov, 1970) and sulfide-silver (Timm, 1958) methods have been used widely for the histochemical determination of zinc. A common shortcoming of these methods is the impossibility of successive determination of enzyme activity in a single section. Methods of luminescent zinc determination (Torontsev and Yeshchenko, 1970) have considerable advantages in this regard. Hence, we have used the luminescent reaction in the present study.

Material and method. The material consisted of the following: the pancreas and submaxillary gland of the rabbit, the small intestine and prostate gland of the rat, and the retina of the cat eye. The animals were killed by air embolism. After removal, the organs were used to obtain frozen sections and fixed according to Timm. Freshly frozen sections 10 - 20 microns thick, prepared in a cryostat, were fixed in an 0.01% acetone solution of one of the substances in the 8-(arensulfonylamino)-quinoline series (8SQ). Fixation time was 30 minutes at 4° C.

The tissues were fixed according to Timm (3 to 6 hours at 4° in 70% alcohol, saturated with hydrogen sulfide) and dehydrated in a series of alcohol solutions of increasing concentration: 70% (1 hour), 80% (2 hours), 90% (2 hours), absolute (2 hours). The tissues were then stored in two batches of petroleum ether (15 minutes each), in two batches of paraffin (1 hour each), and then embedded in paraffin.

Paraffin sections 5 - 10 microns thick were fixed on cover glasses, dehydrated with an ash-free filter, deparaffinated in two batches of petroleum ether (1 minute each), dried in air, and processed with a 0.01% acetone solution of one of the 8SQ substances.

The following 8SQ series compounds were used: 8-(n-toluolsulfonylamino)-quinoline, 8-(benzosulonylamino)-quinoline, 8-(methanosulfonylamino)-quinoline. The fluorescent reaction was performed in reflected ultraviolet radiation by means of the 01-17

luminescence apparatus. The UFS-1 and UFS-3 light filters were used to excite the luminescence. A light filter of ZhS-3 glass was used for protection (as the ocular).

The acid phosphatase activity was determined using frozen and paraffin sections prepared by the modified method of Goldberg and Barka (1962). As the substrate the authors used α-naphthyl phosphate (2 mg/ml of incubation medium), --* diazonium --* ($3.6 \cdot 10^{-3}$ M). The frozen sections were incubated for 45 to 60 minutes; the paraffin sections, 2 to 3 hours. The histochemical reaction to acid phosphatase was studied in the bright field of the microscope.

When the frozen sections were examined under the dark field of the microscope the tissue structure was clearly visible. For example, the A cells of the islands of Langerhans in the pancreas of the rabbit shone brightly with a silvery blue color, while the B cells were yellow. By using the dark field condenser on the microscope, it was possible to see simultaneously in the section a quite clear structure of the tissue and the luminescent reaction. Photographing the sections in the three stages of the study (dark field picture, luminescent reaction, and --* chemical reaction to the enzyme) and subsequent comparison of the pictures made it possible to establish a precise location of the zinc and enzyme in the cells.

Results and evaluation. In those sections which were processed with 8SQ substances, a clear yellowish-green fluorescence was seen in those areas that were occupied by zinc. At high magnification (above 280X) the cellular cytoplasm could clearly be seen to contain granules in the form of small bright grains. The nuclei failed to luminesce and had the appearance of dark spherical formations. Acid phosphatase activity was observed in the form of a fine granulation of orange-raspberry color, detected at high magnification.

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*Translators Note: Illegible in the foreign text.

Cytochemical reaction to zinc (a) and acid phosphatase (b) in a single section of rabbit pancreas in an island. Fixed in acetone solution of 8-(n-toluosulfonylamino)-quinoline. Objective: 40X; ocular: 10X

The reaction to zinc and enzyme in the pancreas was marked in the islands of Langerhans and very mild in the exocrine tissue. In the A cells the granulation filled the cytoplasm uniformly, while in the B cells it was concentrated in the apical regions (see the figure). In the submaxillary gland both reactions were observed in the cells of the albuminous crescents; in the small intestine, in the cells in the bottom of the intestinal crypts; in the prostate gland, in the epithelium of the terminal portions; in the retina, in the intermediate substance between the goblet cells, villi, and gangliose cells.

Fixing the frozen sections in solutions of 8SQ as well as fluorochroming of the paraffin sections does not have a significant effect on the activity of acid phosphatase. However, in the process of fixing according to Timms and embedding the tissues in paraffin, a considerable part of the activity of the enzyme is lost. Nevertheless, the paraffin-section method does have its advantages: the possibility of obtaining quite thin sections and a better structuring of the tissue.

The methods we have proposed, which make it possible to compare the intracellular distribution of zinc and the activity of acid phosphatase, open up the possibility of explaining the topochemical interactions between them.

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